REMARKS

Claims 22-33 are under examination. Applicants thank Examiners Szperka and Ewoldt for the interview held with applicants' representative on November 14, 2006. The issues raised during the interview are discussed below.

Cross-Reference to Related Applications

Applicants have amended the specification to indicate that the present application claims benefit of U.S. Provisional Application Serial No. 60/261,405 filed on January 11, 2001. This application is also a continuation-in-part of U.S. Application Serial No. 10/030,522 filed December 31, 2001, which is the U.S. National Phase application of PCT/EP00/06677 filed July 13, 2000. PCT/EP00/06677, in turn, claims benefit of U.S. Provisional Application Serial No. 60/143,891, filed July 14, 1999 and GB 9916450.1 filed July 14, 1999. (A copy of applicants' priority claim as filed January 11, 2002 is attached as Appendix A.)

Claim Rejections under 35 U.S.C. § 112, first paragraph

All claims stand rejected under 35 U.S.C. § 112, first paragraph on enablement and written description grounds. For the following reasons, these rejections should be withdrawn.

As an initial matter, applicants note that claim 22 has been amended to relate to "A method for treating a mammal at risk of developing Systemic Inflammatory Response Syndrome or suffering from Systemic Inflammatory Response Syndrome..." Claim 22, as amended, reads:

> 22. A method for treating a mammal at risk of developing Systemic Inflammatory Response Syndrome or suffering from Systemic Inflammatory Response Syndrome by administering a partial inhibitor of factor VIII to the said mammal which is a monoclonal antibody against factor VIII or an antigen binding fragment of said monoclonal antibody, said antibody or fragment being able to recognize epitopes located in the C1 domain of factor VIII.

Support for the present amendment is found throughout the specification. For example, applicants point out that the specification at page 1 (lines 25-27) states; "Systemic inflammation is the possible endpoint of a number of clinical conditions including pancreatitis, ischemia, multiple trauma and tissue injury, haemorrhagic shock, immune-mediated organ injury and infection." The skilled artisan would recognize that such patients are at risk of developing SIRS and therefore would understand that such patients would benefit from prophylactic treatment according to the claimed method. Applicants, at page 24 (lines 1-3), further note that the methods of the invention are useful for "prophylactic or therapeutic treatment." No new matter has been added by the present amendment.

In connection with the enablement rejection, the Office asserts that the practice of the invention would require undue experimentation "to make the genus of antibodies recited in the instant claims" and that "prevention [of SIRS] requires the recited method to be completely effective in all patients at all times." For the following reasons, these grounds of rejection should be withdrawn.

With respect to the prevention issue, applicants note that this basis of the enablement rejection may be withdrawn in view of the present claim amendment which specifies that the method is directed to treating a patient at risk of developing SIRS or suffering from SIRS, an amendment suggested by the Office during the interview of November 14, 2006.

Turning to the enablement of the genus of antibodies recited in the instant claims, applicants again note that such antibodies or antigen-binding fragment of such monoclonal antibodies, which are partial inhibitors of factor VIII, are routinely produced absent undue experimentation. First, applicants again point out that, as detailed in the application at Example 1 and Example 5 respectively under the headings "Production of Monoclonal Antibodies Derived from Hemophilia A Patients" and "Monoclonal Antibodies Derived from Hemophilia A Patients Partially Inhibit Thrombin Formation in vitro," Krix-1 was obtained by a cloning procedure which starts from B lymphocytes obtained from patients suffering from Hemophilia, more particularly from patients having an impaired factor VIII function. Such patients are then administered a sufficient

amount of wild-type protein to elicit an immunological response, i.e. the production of antibodies directed against wild-type factor VIII. After isolation of the B lymphocytes from these patients, those cells producing antibodies with the desired properties are selected.

Indeed, applicants' specification at page 17 (line 34) through page 8 (line 14) teaches production of partial inhibitors:

> *Human monoclonal antibodies of the desired specificity and characteristics are produced by transformation of B lymphocytes obtained from the peripheral blood of patients suffering from hemophilia A or acquired hemophilia. [...] In order to elicit a more specific immunological response, patients are sought who have an impaired function of a physiologically active protein, e.g. factor VIII. By "impaired" is meant that some residual function is available but that this is less than is known for the wild-type of the same protein. A comparison between the self-protein and the wild-type protein should exhibit a difference in the two proteins, preferably in a region or domain which is of interest. The difference may be a deletion or a substitution of one or more amino acids with others. The patients are then administered enough of the wild-type protein to elicit an immunological response. Then, B-lymphocytes are extracted from the patients and selected based on the production of antibodies which have desirable properties. Although reference is made to "patients" above, the method in accordance with this embodiment may be applied generally to mammals. The above procedure results in a greater chance of obtaining antibodies which target the domain containing the defect.*

Accordingly, applicants' specification teaches a method which specifically ensures the generation of partial inhibitory antibodies. Indeed, patients having a partially impaired physiological function of factor VIII are described as patients in which some residual factor VIII activity is present, as a result of a mutation in the domain of interest (here the C1 domain). The mutation is one which does not completely inactivate factor VIII function. The fact that factor VIII is only partially impaired in most of these patients is because complete impairment of factor VIII function significantly reduces the survival rate. When the wild-type factor VIII protein is administered to these subjects, antibodies

are generated within this patient against the corresponding wild-type epitope corresponding to this mutation (as this is recognized as 'foreign'). Similar to the effect of the presence of the mutation at this position in factor VIII, the antibodies directed against this epitope of factor VIII will result in only partial inhibition of factor VIII activity.

To further support applicants' position that obtaining antibodies from "Hemophilia A Patients" as described in the specification is a routine matter, applicants note that patients were known in the scientific literature to generate polyclonal antibodies capable of inhibiting factor VIII function. Indeed, it was described as early as 1982, that polyclonal antibodies inhibiting the co-factor activity of factor VIII can be classified as type I or type II inhibitors according to their capacity to inhibit factor VIII either completely (type I) or only partially (type II) (see, for example, Gawryl et al., Blood (1982) 60:1103; copy enclosed as Appendix B (see also Information Disclosure Statement initialed April 5, 2004; copy enclosed as Appendix C)). The present invention accordingly demonstrates that such partial inhibitory antibodies can be generated most particularly in patients in which the Hemophilia is a result of partial impairment of factor VIII activity due to a mutation in the C1 domain of factor VIII. Again, obtaining a partial inhibitory antibody of factor VIII cannot be considered undue experimentation.

Finally, in connection with the enablement rejection, applicants again direct the office's attention to the Declaration of Dr. Jean-Marie Saint-Remy filed October 20, 2005 (copy enclosed as Appendix D). Here Dr. Saint-Remy makes clear that the method as described in the application as filed could indeed be used to reproduce a partial inhibitor, using the production of the antibody termed "RHD5," as an example. More particularly paragraphs 11 and 12 of that declaration provide data that describe the method used for obtaining antibody RHD5, which corresponds to the method described in the application. Under paragraphs 13 to 16, the partial inhibitory activity of this antibody and its ability to compete with Krix-1 is detailed. Such data indicate that antibodies falling within the scope of the claims are produced using routine methods and absent undue experimentation following the methods described in the application.

The Office further asserts that all of the claims are unpatentable under § 112, first paragraph, because they lack an adequate written description. Here, relying on the

Federal Circuit's opinion in *The Regents of the University of California v .Eli Lilly* (43 USPQ2d 1398-1412) 19 F.3d 1559, the Office, in essence, asserts that because the specification discloses only one member of the genus of antibodies recited in the independent claim a skilled artisan would reasonably conclude that applicant was not in possession of the recited genus of antibodies at the time the application was filed. For the following reasons, this rejection should also be withdrawn.

Applicants again direct the Office's attention to the Gawryl reference which describes a class of factor VIII antibodies known in the scientific literature at the time the application was filed that do not completely inactivate factor VIII.

Applicants next submit that administration of "a partial inhibitor of factor VIII to the said mammal which is a monoclonal antibody against factor VIII or an antigen binding fragment of said monoclonal antibody, said antibody or fragment being able to recognize epitopes located in the C1 domain of factor VIII" as recited broadly in the invention would naturally occur to one skilled in the art reading the description. Applicants' description is clearly not limited to KRIX-1. Broader claim language, in this case, is permissible because the description of the use of a partial inhibitor of factor VIII throughout entire specification would immediately convey to any skilled person that applicant invented a method that involves administration of a partial inhibitor of factor VIII which binds to the C1 domain of factor VIII. The Gawryl reference provides additional evidence of the knowledge of one skilled in the art of anti-factor VIII, and as such supports applicants' position that to the ordinary skilled worker applicants' specification would be understood to include a class of antibodies that did not completely inactivate factor VIII as such partial inhibitors. Accordingly, under the facts of this case, applicants assert that, in view of the broad description of using partial inhibitors of factor VIII that bind to the C1 domain and the results obtained using one such antibody, KRIX-1, and the fact that additional antibodies that did not completely inactivate factor VIII were known in the art, that the scope of the pending claims would be so readily recognized by one of ordinary skill in the art. Accordingly, on this basis alone, the written description rejection should be withdrawn.

Moreover, applicants note that, the Federal Circuit, in *Falkner v. Inglis*, 448 F.3d 1357, 79 USPQ2d 1001 (Fed. Cir. May 26, 2006) has stated:

> [I]t is the binding precedent of this court that Eli Lilly does not set forth a per se rule that whenever a claim limitation is directed to a macromolecular sequence, the specification must always recite the gene or sequence, regardless of whether it is known in the prior art. See Capon, 418 F.3d at 1357 ("None of the cases to which the Board attributes the requirement of total DNA re-analysis, i.e., Regents v. Lilly, Fiers v. Revel, Amgen, or Enzo Biochem, require a redescription of what was already known."). Thus, "[w]hen the prior art includes the nucleotide information, precedent does not set a per se rule that the information must be determined afresh." Id. at 1358. Rather, we explained that:
>
> > The descriptive text needed to meet these requirements varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science.

The court further stated:

> Indeed, a requirement that patentees recite known DNA structures, if one existed, would serve no goal of the written description requirement. It would neither enforce the quid pro quo between the patentee and the public by forcing the disclosure of new information, nor would it be necessary to demonstrate to a person of ordinary skill in the art that the patentee was in possession of the claimed invention. As we stated in Capon, "[t]he 'written description' requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution." Id at 1358. Indeed, the forced recitation of known sequences in patent disclosures would only add unnecessary bulk to the specification. Accordingly we hold that where, as in this case, accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences (here "essential genes"), satisfaction of the written description requirement does not require either the recitation or incorporation by reference [note omitted] (where permitted) of such genes and sequences.

Applicants submit that the present specification provides a written description of the presently claimed invention in sufficient detail to satisfy the standard set by the Federal Circuit in *Falkner,* 448 F.3d 1357, 79 USPQ2d 1001. Like the situation in *Falkner*, where that the written description of a genus of poxvirus DNA was supported by mentioning vaccinia virus, a poxvirus, applicants' disclosure of the use of partial inhibitors of factor VIII and the description of the use of the KRIX-1 antibody or an antigen-binding fragment thereof supports the written description of the genus of partial inhibitors encompassed by the present claims.

Finally, in connection with the assertion that "applicants' specification does not disclose the precise epitope recognized by the recited genus of antibodies, nor does it identify the structure an antibody must comprise in order to comprise the recited function", applicants note the following. Applicants point out that they are merely claiming a class of antibodies that recognize the C1 domain of factor VIII. Applicants also point out that the Office does not question that the C1 domain is a fully characterized antigen, in view of its structure, formula, chemical name, or its physical properties. Indeed, the USPTO Guidelines are persuasive authority for the proposition that a claim directed to "any antibody which is capable of binding to antigen X" would have sufficient support in a written description that disclosed "*fully characterized* antigens." Synopsis of Application of Written Description Guidelines, at 60, *available at* http://www.uspto.gov/ web/menu/written.pdf (last visited December 26, 2006) (emphasis added). Although the present claims are directed to methods of using antibodies that recognize the C1 domain of factor VIII, the same principles apply in the present situation, and on this basis too the written description rejection should be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a Petition to extend the period for replying to the final Office action for two (2) months, to and including February 26, 2007.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 26 February 2007

James D. DeCamp
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APPENDIX A

0522-1769.1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE THE APPLICATION OF	)	Examiner:
Jaquemin, Marc G. & Saint-Remy, Jean-Marie R.	)	Group Art Unit:
SERIAL NO.: To Be Assigned	)	
FILED: January 11, 2002	)	
FOR: METHOD AND PHARMACEUTICAL COMPOSITION FOR PREVENTING AND/OR TREATING SYSTEMIC INFLAMMATORY RESPONSE SYNDROME	)	

Honorable Director of
Patents and Trademarks
Washington, D.C. 20231

PRIORITY CLAIM and ASSERTION OF SMALL ENTITY STATUS

Dear Sir:

Applicants hereby claim priority benefits based upon the co-pending U.S. national phase application, filed December 31, 2001 and titled "Ligands for Use in Therapeutic Compositions for the Treatment of Hemostasis Disorders," which in turn was based upon International Application Serial No. PCT/EP00/06677 having an international filing date of July 13, 2000, claiming priority from GB 9916450.1 and US 60/143,891. Applicant's further claim priority of co-pending U.S. Provisional Application Serial No. 60/261,405 filed on January 11, 2001.

Applicants hereby assert small entity status under 35 CFR § 1.27 with respect to the payment of fees for prosecution of the instant U.S. application.

The sequence listing information in written form (pages 1-15) submitted herewith and the sequence listing information in computer readable format (two 3.5" diskettes labeled Copy 1 and Copy 2) submitted herewith are identical.

Respectfully Submitted,

January 11, 2002

Date

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Inactivation of Factor VIII Coagulant Activity by Two Different Types of Human Antibodies

By Maria S. Gawryl and Leon W. Hoyer

Human antibodies that inactivate factor VIII procoagulant activity (VIII:C) are heterogeneous in their kinetic properties. We report here the properties of four type I and four type II antibodies classified according to Biggs et al. Type I antibodies have second-order inactivation kinetics and completely destroy VIII:C when present in high concentration; type II antibodies have more complex kinetics and do not completely inactivate VIII:C even when tested undiluted. The latter properties correspond to the in vivo finding in some patients that there is detectable VIII:C, even though there is also a significant inhibitor titer. It has been suggested that the antibody-antigen complex in these patients retains some VIII:C activity. This is unlikely, however, since protein-A-Sepharose (PAS) did not adsorb any VIII:C activity from mixtures of type II antibodies with normal human plasma. An alternate possibility, reduced VIII:C inactivation due to a steric effect of the factor-VIII-related protein (VIIIR, von Willebrand factor), appears to be a more important factor, since three of four type II antibodies had inactivating properties like type I antibodies when they were tested with separated VIII:C instead of plasma. Although the fourth type II antibody did not completely inactivate separated VIII:C, the residual coagulant activity was adsorbed from this mixture by PAS. These data indicate that type II anti-VIII:C react with different antigenic determinants than type I antibodies and that these determinants are partially blocked in the factor VIII complex by VIIIR.

ANTIBODIES TO FACTOR VIII develop in approximately 5%–20% of patients with severe classic hemophilia who require repeated transfusions.[1] They also occur spontaneously as autoantibodies in postpartum women, in patients with autoimmune diseases, and in elderly individuals with no apparent abnormality.[2] These IgG antibodies inactivate human factor VIII procoagulant activity (VIII:C) and do not react with human factor-VIII-related protein (VIIIR, von Willebrand factor).[3,4]

The inactivation of VIII:C by these human antibodies is time and temperature dependent.[3,4] When carefully studied, the inactivation pattern is not uniform, however, and two types of antibodies have been distinguished by kinetic analysis. Type I antibodies, in sufficient quantities, completely inactivate VIII:C and there is a linear relationship when the logarithm of residual VIII:C activity is compared to the antibody concentration.[3] In contrast, type II antibodies do not completely inactivate VIII:C, even when undiluted. VIII:C inactivation by type II antibodies has a different kinetic pattern as well, with a nonlinear (complex) relationship of residual VIII:C and antibody concentration.[5] These properties of type II antibodies may be responsible for the observation in some patients that small amounts of VIII:C can be detected even though an inhibitor is present.[3,6] It has been suggested that the antibody–antigen complexes in these patients retain VIII:C activity[3] or that there is a spontaneous dissociation of relatively weak immune complexes.[3] To examine these hypotheses, type I and type II human anti-VIII:C have been tested with plasma factor VIII complexes and with separated VIII:C. Both standard inhibition assays and adsorption studies have been carried out.

MATERIALS AND METHODS

Factor VIII Measurements

Factor VIII procoagulant activity (VIII:C) was measured by a one-stage method using factor-VIII-deficient human plasma as substrate.[7] Factor VIII procoagulant antigen (VIII:CAg) was measured by an immunoradiometric assay using ^{125}I-labeled Fab' prepared from a type I human anti-VIII:C plasma.[7] Factor-VIII-related antigen (VIIIR:Ag) was determined by an immunoradiometric assay using a rabbit antibody.[7] The standard (1 U/ml) for all factor VIII measurements was pooled normal human plasma, prepared as previously described.[7]

Anti-VIII:C Measurements

Inhibition of VIII:C procoagulant activity was determined by incubating equal volumes of pooled normal human plasma or separated VIII:C[7] with a dilution of antibody plasma for 2 hr at 37°C. The residual VIII:C activity was then measured and in some studies the antibody titer was expressed in Bethesda units.[8] This value was the reciprocal of the antibody plasma dilution that inactivated 50% of the VIII:C activity during the 2-hr incubation. The value for each antibody plasma was the mean of assays done at five different plasma dilutions.

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Presented in part at the 23rd Annual Meeting of the American Society of Hematology, San Antonio, Texas, December 2, 1981 (Blood, 58 (Suppl 1): 216a, 1981, abstr).

0006-4971/83/6005-0007$01.00/0

Human Anti-VIII:C

Eight antibody plasmas that inactivated VIII:C were studied in detail. One type I antibody was obtained from a patient with no previous hemostatic disorder (Ab1). The other three type I antibody plasmas were obtained from patients with severe classic hemophilia who had been repeatedly transfused (Ab2–4). All of the four type II antibodies (Ab5–8) occurred as autoantibodies. These plasma samples had been stored at −70°C for 0.5–13 yr before these studies. The inhibitor plasmas were obtained through the helpful cooperation of Drs. E. G. D. Tuddenham, J. Miller, and H. S. Weiss. One plasma (Ab8) was purchased from George King Biomedical, Inc. (Overland Park, Kans.).

The classification of antibodies as type I or type II followed the criteria of Biggs and Macfarlane.[16] The relationship of residual VIII:C activity (logarithmic scale) to antibody concentration was determined after a 2-hr incubation with normal plasma at 37°C.

Adsorption of Antibodies and Immune Complexes With Protein-A-Sepharose

Antibody and VIII:C mixtures were adsorbed with protein-A-Sepharose (PAS) (Pharmacia Fine Chemicals, Piscataway, N.J.) after a 2-hr incubation at 37°C. Exactly PAS (3 ml of a 20% suspension of PAS beads in saline) was added to 0.6 ml of the mixture and the incubation continued at 37°C for 15 min. The PAS beads were then removed by centrifugation and the supernatant fluid examined for residual VIII:C. The maximum IgG/PAS ratio in these experiments (10 mg IgG/ml PAS) was well below the capacity of the beads.

In some experiments, human anti-VIII:C antibodies were immobilized by adsorption to PAS before being mixed with VIII:C. After the beads had been incubated with the antibody-containing plasma for 2 hr at room temperature, the beads were washed 3 times with large volumes of barbital-buffered saline (0.125 *M* NaCl, 0.015 *M* barbital, 0.010 *M* sodium barbital, pH 7.3) (BBS). The supernatant fluid was examined in each experiment and it contained less than 2% of the anti-VIII:C activity.

The volume of beads was kept constant in these experiments by employing mixtures of antibody–PAS beads and untreated Sepharose 4B–CL. Incubated normal human plasma or partially purified VIII:C[H] was incubated with an equal volume of the antibody–beads for 2 hr at 37°C and the residual VIII:C was determined in the supernatant fluid after the beads had been removed by centrifugation. PAS beads saturated with normal human plasma IgG served as a control reagent for these studies.

The amount of anti-VIII:C adsorbed to PAS beads was calculated with the assumption that all plasma antibody was bound. This assumption was verified in several studies in which the adsorbed IgG was eluted from washed PAS–antibody beads at pH 2.4. A glycine–NaCl buffer (0.05 *M* glycine, 0.1 *M* NaCl, 0.02% sodium azide) was used at a buffer:bead ratio of 5:1 (v/v), the beads removed by centrifugation (7400 *g*) for 20 min at room temperature, and the supernatant fluid added to 1/40 volume borate buffer (0.1 *M* boric acid, 0.01 *M* sodium borate, 0.075 *M* sodium chloride pH 8.4). After dialysis against at least 250 volumes of borate-buffered saline, pH 7.5 (0.016 *M* boric acid, 0.005 *M* sodium hydroxide, 0.150 *M* sodium chloride), the eluate was concentrated to 1 ml by negative pressure ultrafiltration.

The amount of IgG eluted was determined by Laurell immunoelectrophoresis using rabbit antibodies specific for human gamma

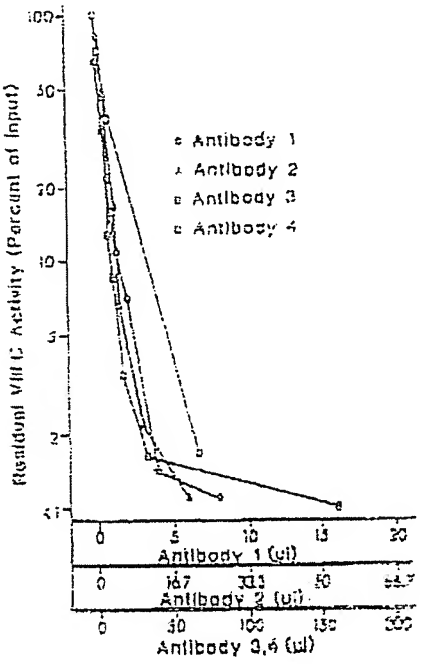

Fig. 1 The inactivation of plasma VIII:C by four type I antibodies. Dilutions of antibody plasma in saline (0.3 ml) and an equal volume of normal plasma were incubated for 2 hr at 37°C prior to the measurement of residual VIII:C activity.

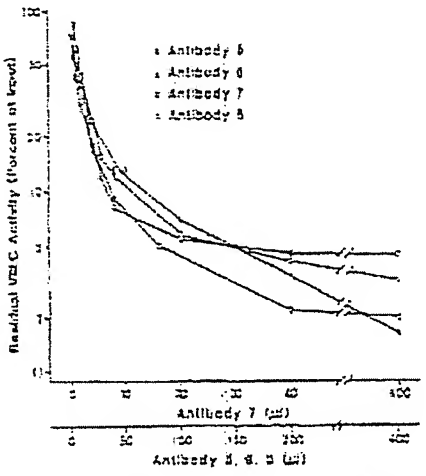

Fig. 2 The inactivation of plasma VIII:C by four type II antibodies. Dilutions of antibody plasma in saline (0.3 ml) and an equal volume of normal plasma were incubated for 2 hr at 37°C prior to the measurement of residual VIII:C activity.

Table 1. Properties of Human Anti-VIII:C

Antibody	Source	Titer (Bethesda Units/ml)	Class*
1	Autoantibody	800	1
2	Hemophiliac	218	1
3	Hemophiliac	78	1
4	Hemophiliac	14	1
5	Autoantibody	60	2
6	Autoantibody	36	2
7	Autoantibody	201	2
8	Autoantibody	102	2

*Determined by reaction kinetics and the use of VIII:C inhibitor.[12]

heavy chains.[9] The anti-VIII:C titer of the eluted IgG was determined in the same way as the plasma samples.[1]

RESULTS

The VIII:C inactivating properties of 8 human antibodies were characterized by the method of Biggs and coworkers.[1,6] Type I antibodies (Ab1–4), at high concentrations, inactivated more than 98% of the VIII:C in a manner consistent with second-order kinetics, resulting in a linear inactivation response (Fig. 1). Undiluted type II antibodies (Ab5–8) did not completely inactivate plasma VIII:C, and the VIII:C inactivation graph had a curvilinear pattern (Fig. 2). The source, titer, and inactivation patterns of the 8 antibodies are given in Table 1.

The basis for nonlinear inactivation by type II antibodies was investigated by incubating plasma–antibody mixtures with protein-A-Sepharose (PAS) to remove most IgG and any immune complexes formed by IgG_1, IgG_2, or IgG_4 antibodies. Preliminary experiments established that all of the anti-VIII:C activity was adsorbed from the inhibitor plasma when a sufficient quantity of PAS was added.

In control studies, the adsorption of type I antibody–plasma mixtures with PAS had minimal effect on VIII:C inactivation (Fig. 3). Similarly, additional VIII:C inactivation was not noted when type II antibody–plasma mixtures were adsorbed with PAS. Typical data are given in Fig. 4 (Ab8) and Fig. 5 (Ab5). Thus, the nonlinear and incomplete VIII:C-inactivating characteristics of type II anti-VIII:C seen when type II antibodies are incubated with plasma cannot be attributed to the formation of immune complexes that retain VIII:C activity.

The potential role of another factor, steric interference by the factor-VIII-related protein (VIIIR, von

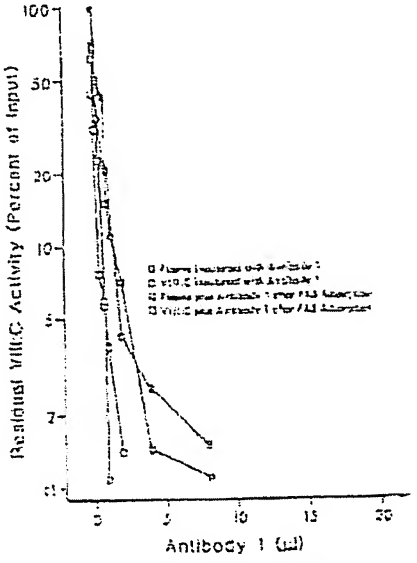

Fig. 3. VIII:C inactivation by antibody 1. Dilutions of this type I antibody in 0.3 ml saline were tested with 0.3 ml normal human plasma (●) or with separated VIII:C (○). In parallel experiments, 0.5 ml PAS was added to similar mixtures after the initial 2-hr incubation. The residual VIII:C activity was then determined after the PAS beads had been removed by centrifugation from mixtures of Ab1 with normal human plasma (▲) or with separated VIII:C (△). Similar patterns were identified using Ab2, 3, and 4.

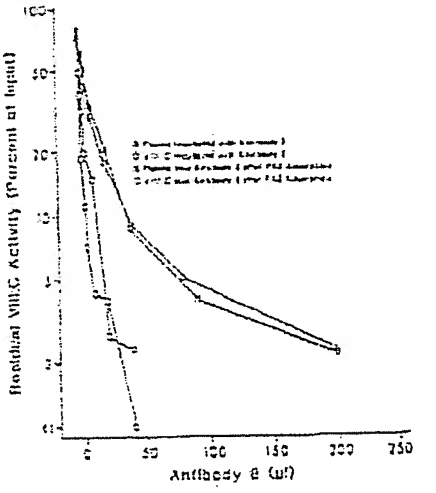

Fig. 4. VIII:C inactivation by antibody 8. Dilutions of this type II antibody in 0.3 ml saline were tested with 0.3 ml normal human plasma (●) or with separated VIII:C (○). In parallel experiments, 0.3 ml PAS was added to similar mixtures after the initial 2-hr incubation. The residual VIII:C activity was then determined after the PAS beads had been removed by centrifugation from mixtures of Ab8 with normal human plasma (▲) or with separated VIII:C (△). Similar patterns were identified using Ab6 and 7.

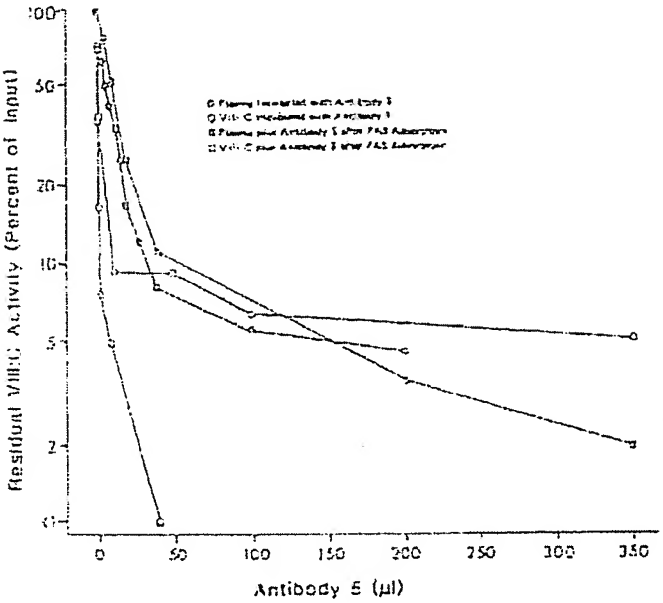

Fig. 5. VIII:C inactivation by antibody 5. Dilutions of this type II antibody in 0.3 ml saline were tested with 0.3 ml normal human plasma (O) or with separated VIII:C (O). In parallel experiments, 0.5 ml PAS was added to similar mixtures after the initial 2-hr incubation. The residual VIII:C activity was then determined after the PAS beads had been removed by centrifugation from mixtures of Ab5 with normal human plasma (O) or with separated VIII:C (O).

Willebrand factor), was also considered. In these studies, type I and type II anti-VIII:C were tested with partially purified VIII:C that had VIII:C to VIIIR:Ag ratios greater than 990:1—in contrast to the 1:1 ratio (by definition) in normal plasma [13]

Type I antibodies had similar properties when tested with separated VIII:C, and the inactivating capacity was only slightly greater than that observed with intact plasma (Fig. 3). Subsequent adsorption of the antibody–VIII:C mixture with PAS had no further effect on the amount of residual VIII:C. Thus, VIIIR did not affect the VIII:C-inactivating properties of the 4 type I antibodies.

In contrast, three of the type II antibodies (Ab6–8) inactivated much more VIII:C when it had been separated from VIIIR (Fig. 4). No further augmentation of antibody potency was observed in these experiments if the antibody–VIII:C mixture was adsorbed with PAS. The other type II antibody, Ab5, retained type II characteristics when tested with separated VIII:C, and its properties were unchanged from those observed with whole plasma (Fig. 5). The adsorption of immune complexes by PAS removed VIII:C activity in this case, however. Thus, VIIIR inhibited VIII:C binding by each of the four type II antibodies. In three cases the antibodies had type I properties when tested with separated VIII:C; in the fourth case (Ab5), the interaction produced an immune complex that retained VIII:C activity

VIII:C Inactivation by Immobilized Antibodies

A second group of experiments were carried out with immobilized type I and type II antibodies. The quantity of type I or type II antibody plasma incubated with PAS was chosen so that there would be approximately 100 Bethesda units of anti-VIII:C adsorbed by each milliliter of PAS, and the amount of bound antibody was verified in each case by testing the supernatant fluid. In control experiments, normal human plasma IgG was adsorbed with PAS in the same way.

Immobilized type I anti-VIII:C had the same properties as did the antibody in solution. Both plasma VIII:C and separated VIII:C were inactivated—presumably by removal from solution—and the dose-response pattern was linear (Fig. 6). In contrast, the four type II antibodies adsorbed *less* VIII:C from plasma when they were bound to PAS (Fig. 7). The immobilized type II anti-VIII:C were potentially reactive, however, for they removed over 98% of the VIII:C activity when incubated with separated VIII:C. This pattern—reduced reactivity with *plasma* VIII:C and increased reactivity with *separated* VIII:C—was consistent for each of the four immobilized type II anti-

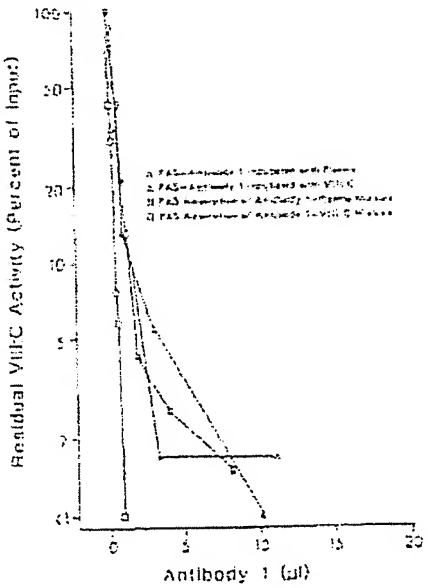

Fig. 6. VIII:C inactivation by antibody 1. This figure compares the effect of PAS immobilized Ab1 incubated with plasma or separated VIII:C for 2 hr at 37°C and Ab1 incubated with an VIII:C source for 2 hr at 37°C prior to the addition of PAS. Similar patterns were obtained with Ab2, 3, and 4.

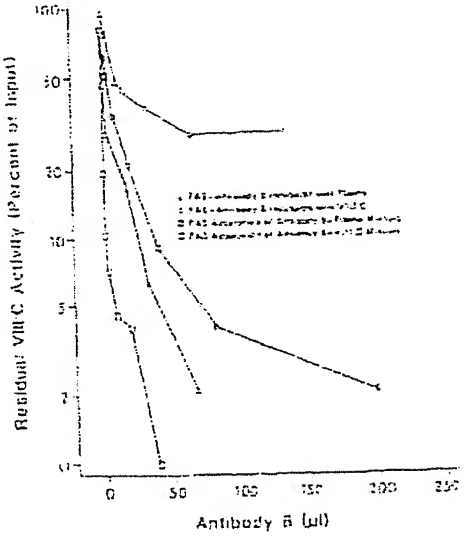

Fig. 7. VIII:C inactivation by antibody 6. This figure compares the effect of PAS immobilized Ab6 incubated with plasma or separated VIII:C for 2 hr at 37°C and Ab6 incubated with an VIII:C source for 2 hr at 37°C prior to the addition of PAS. Similar patterns were obtained with Ab5, 6, and 7.

bodies. As expected, normal human IgG bound to PAS had no effect on either plasma or separated VIII:C, and 95% ± 7% (1 SD) residual activity was measured in three studies.

Both type I and type II antibodies could be eluted from the PAS with glycine-buffered saline, pH 2.5. Measurement of anti-VIII:C activity recovered in this way verified the calculated amount of antibody that had been immobilized.

The studies with immobilized type II antibodies strongly suggested that VIIIR partially blocks the interaction of type II anti-VIII:C with VIII:C determinants. This conclusion was supported by the demonstration that VIIIR in hemophilic plasma inhibited in a dose-dependent manner the inactivation of separated VIII:C by immobilized type II antibodies (Fig. 8). Hemophilic plasma VIIIR had no effect on the properties of an immobilized type I antibody (Ab1, Fig. 8).

The Effect of Type I and Type II Anti-VIII:C on VIII:CAg and VIIIR:Ag Measurements

Residual VIII:CAg and VIIIR:Ag were measured in each of the studies described above. The residual VIII:CAg levels were similar to most of the VIII:C values, but higher values were noted after some adsorptions. Representative data for a type I antibody (Ab1) and a type II antibody (Ab6) are given in Tables 2 and 3. The immobilized type I and type II antibodies did not remove any VIIIR from plasma (Tables 2 and 3) and the residual VIIIR:Ag content in 10 separate experiments was 97% ± 4% (1 SD) of that in plasmas incubated with control beads. The separated VIII:C

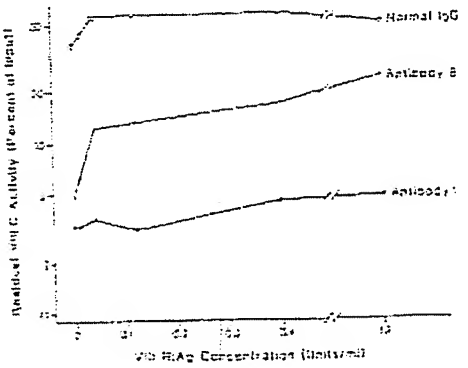

Fig. 8. The effect of hemophilic plasma on the inactivation of separated VIII:C by PAS-immobilized Ab1 (type I), Ab6 (type II), and normal IgG. Dilutions of hemophilic plasma made in severe von Willebrand's disease plasma (0.1 ml) were incubated for 2 hr at 37°C with 0.1 ml of immobilized antibody beads and 0.2 ml of separated VIII:C.

Table 2. Protein-A-Sepharose Adsorption of Anti-VIII:C Incubated With Plasma or Separated VIII:C

		Residual Values				
		Antibody Plus Plasma			Antibody Plus Separated VIII:C	
Antibody	Volume* (µl)	VIII:C (U/ml)	VIII:CAg (U/ml)	VIII:Ag (U/ml)	VIII:C (U/ml)	VIII:CAg (U/ml)
Ab1	1.5	0.04	0.65	0.48	<0.01	0.30
	0.75	0.21	0.17	0.37	<0.01	0.17
	0.38	0.51	0.30	0.37	0.08	0.25
Ab5	150	0.03	0.15	0.48	<0.01	0.06
	30	0.13	0.25	0.49	0.02	0.05
	7.5	0.47	0.64	0.47	0.14	0.37
Buffer	300	0.48	0.55	0.50	0.46	0.47

*Volume of antibody plasma in total volume of 300 µl. To this was added 300 µl of either normal plasma or separated VIII:C (1.0 U/ml). The mixture was incubated for 2 hr at 37°C and the IgG adsorbed with 600 µl Protein A-Sepharose.

†At each VIII:C source was diluted 1:2 with either plasma antibody plasma or buffer. 0.5 U/ml indicates no loss or inactivation.

had very little VIII:R.Ag (<0.1 U/ml) prior to the adsorption.

DISCUSSION

The inactivation properties of type I and type II human anti-VIII:C have been compared in this study so that the basis for the distinction could be clarified. By studying the ability of protein-A-sepharose to remove residual VIII:C from solutions containing antigen–antibody complexes, we were able to show that four type II antibodies do not form immune complexes that retain VIII:C activity when they are tested with normal human plasma. Similar inactivation data and residual VIII:C values were obtained before and after protein-A-sepharose adsorption of mixtures containing plasma and type II antibodies. If the type II antibodies were immobilized on protein-A-sepharose before being exposed to plasma, 10%–40% less plasma VIII:C was inactivated (Fig. 7).

In these studies, the less effective VIII:C inactivating properties of type II antibodies appeared to be due to steric inhibition by the VIII:R present in factor VIII complexes. This conclusion was based on the observation that type II antibodies inactivated partially purified VIII:C—free of VIII:R—in the same way as do type I antibodies incubated with plasma. Not all type II antibodies behaved identically, however, for one of them (Ab5) had the same characteristics when tested with separated VIII:C or with plasma (Fig. 5). All VIII:C was removed from the Ab5–VIII:C mixture by protein-A-sepharose, however, while the addition of PAS had no effect on Ab5–plasma mixtures. These results indicate that Ab5 reacts with VIII:C at a site different from that bound by the other type II antibodies. In the case of Ab5, the immune interaction is prevented by VIII:R, but the antigen–antibody complex formed in the absence of VIII:R retains VIII:C activity. Unless the complex is removed from solution, as by adsorption with PAS, Ab5 only inactivates part of the VIII:C activity.

The conclusion that type II antibodies recognize VIII:C antigens separate from the procoagulant site was supported by inhibition experiments in which VIII:R was added back to separate VIII:C (Fig. 8). VIII:C inactivation of PAS–Ab5 was inhibited in a dose-dependent manner by hemophilic plasma.

We conclude that the different kinetic properties of the two kinds of human anti-VIII:C are due to the different kinds of antigenic determinants with which they react.[6] Type I antibodies appear to interact with a group of antigenic determinants near the part of the molecule responsible for procoagulant activity. In contrast, type II antibodies recognize determinants remote from this region, and they are partially inhibited when

Table 3. The Effect of Immobilized Anti-VIII:C on Plasma and Separated VIII:C

		Residual Values					
	Incubated With Plasma				Incubated With Separated VIII:C		
Antibody	Volume* (µl)	VIII:C (U/ml)	VIII:CAg (U/ml)	VIII:Ag (U/ml)	Volume* (µl)	VIII:C (U/ml)	VIII:CAg (U/ml)
Ab1	10	<0.01	0.11	1.15	10	0.02	0.06
	1	0.14	0.84	1.13	1	0.13	0.29
	0.1	0.87	0.73	1.11	0.1	0.35	0.48
Ab5	150	0.31	0.65	1.20	134	0.04	0.05
	30	0.55	0.55	1.20	30	0.04	0.31
	10	0.59	0.84	1.00	10	0.49	0.34
Control (normal plasma)	120	0.90	1.20	1.90	134	0.06	1.03

*Volume of antibody or control plasma absorbed to 400 µl protein-A-Sepharose beads (see Methods). These beads were then washed and incubated with 400 µl of normal plasma or separated VIII:C (1.0 U/ml) for 2 hr at 37°C.

†The beads were removed from the mixtures by centrifugation and assays done on the supernatant. In this table, 1.0 U/ml indicates no loss or inactivation.

VIII:C is associated with VIIIR in the intact factor VIII complex. This interpretation is consistent with Green's observation that type I antibodies rapidly and completely inactivated the residual VIII:C activity that was left when plasma was incubated with type II antibodies.[9]

It is not certain why type II antibodies partially inhibit VIII:C of normal plasma or why the inactivation–concentration relationship is complex (Fig. 2). This pattern may indicate that there is heterogeneity in the antibody specificity so that some of the antibodies inactivate plasma VIII:C while other antibodies can only react with the separated coagulant protein. Alternatively, and more likely, the heterogeneity in plasma factor VIII may cause some VIII:C to be susceptible to inactivation while other VIII:C is protected by a close interaction with the VIIIR.

Type II antibodies bound to PAS are even less effective in their ability to inactivate plasma VIII:C. In this case, there are potential steric effects produced by both VIIIR and the protein-A-sepharose. As a result, the incomplete VIII:C inactivating properties of type II antibodies are exaggerated when they are bound to PAS (Fig. 7). Similar observations have been reported for rabbit anti-VIII:C immobilized by coupling to agarose.[11] This steric effect was not detected with type I antibodies (Fig. 6).

Thus, the complex inactivating properties of type II antibodies are due to the antigenic determinants with which they react and the steric interference by the VIIIR protein that partially shields the antigens. In addition, one type II antibody formed an immune complex that retained VIII:C activity. Only one of four type II antibodies had this property, however, and it was demonstrable only when the antibody was added to separated VIII:C. None of the type II antibodies formed VIII:C immune complexes which had residual coagulant activity when they were mixed with unfractionated plasma. For this reason, it is still not certain whether patients with type II antibodies retain some VIII:C activity in immune complexes or if they have, in vivo, a heterogeneous population of VIII:C molecules, some of which retain activity because they are protected by VIIIR.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

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Complete If Known	
Application Number	10/044,569
Filing Date	01/11/2002
First Named Inventor	Marc G. Jacquemin & Jean Marie Saint-Remy
Group Art Unit	1644
Examiner Name	
Attorney Docket Number	0522-1769.1

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MH	2	J. BATLLE ET AL. : "Alloantibody from a patient with severe von Willebrand disease inhibits von Willebrand factor-FVIII interaction." ANNALS OF HEMATOLOGY, vol. 75, no. 3, September 1997 (1997-09), pages 111-115, XP 000906715.	
MH	3	J. INGERSLEV ET AL.: "Applications of immunoperoxidase techniques in specificity testing of monoclonal antibodies (Mabs) against von Willebrand factor (vWF)." CLINICA CHIMICA ACTA, vol. 174, no. 1, 1988, pages 65-82, XP000906709.	
MH	4	M. GAWRYL ET AL.: "Inactivation of factor VIII coagulant activity by two different types of human antibodies." BLOOD, vol.60, no.5, November 1982 (1982-11), pages 1103-1109, XP000892192.	
MH	5	B. LY ET. AL.: "Characterization of an antibody to factor VIII in a patient with acquired hemophilia with circulating immune complexes." SCANDINAVIAN JOURNAL OF HAEMATOLOGY, vol. 28, no. 2, February 1982 (1982-02), pages 132-140, XP000892196.	
MH	6	M. JACQUEMIN ET AL.: "Mechanism and kinetics of factor VIII inactivation: Study with an IgG4 monoclonal antibody derived from a hemophilia A patient with inhibitor." BLOOD, vol. 92, no. 2, 15 July 1998 (1998-07-15), pages 496-506, XP000906844.	
MH	7	M JACQUEMIN ET AL.: "A human antibody directed to the factor VIII C1 domain inhibits factor VIII cofactor activity and binding to von Willebrand factor." BLOOD, vol. 95, no. 1, 1 January 2000 (2000-01-01), pages 156-163, XP002150704.	

Examiner Signature	Maher Haddad	Date Considered	4/5/04

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	SAINT-REMY et al.	Art Unit:	1644
Serial No.:	10/044,569	Examiner:	Maher M. Haddad
Filed:	January 11, 2002	Customer No.:	21559
Title:	METHOD AND PHARMACEUTICAL COMPOSITION FOR PREVENTING AND/OR TREATING SYSTEMIC INFLAMMATORY RESPONSE SYNDROME		

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DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. JEAN-MARIE SAINT-REMY

1. I am a named inventor on the above-referenced patent application.

2. I am a Professor at the University of Leuven and an expert in the field of vascular biology. A copy of my curriculum vitae is attached.

3. I have read and understand the Office Actions mailed April 21, 2004 and December 15, 2004.

D)

4. I present in vivo data from an animal model confirming that an antibody, administered according to the method of the claimed invention, is effective against systemic inflammatory response syndrome (SIRS) such as sepsis.

5. The model that was used, i.e., the induction of sepsis by a single bolus injection of lipopolysaccharide ("LPS") in mice, is a well-established animal model for studying septic shock symptoms and testing potential therapeutic agents in septic shock.

6. In this experiment, we used both the KRIX-1 antibody and a deglycosylated form thereof. The antibody KRIX-1 is produced by the cell line named KRIX 1, which, as detailed in the patent application, was deposited with the Belgian Coordinated Collections of Micro-organisms under accession number LMBP 5089CB. As described in the patent specification, the KRIX-1 antibody binds to an epitope in the C1 domain of FVIII and partially inactivates FVIII. To obtain the latter deglycosylated antibody, we modified carbohydrate attachment sites found in the complementarity determining regions of the KRIX-1 antibody. This modified KRIX-1 antibody was named KRIX-1Q, and was found to retain the binding affinity to the antigen of the KRIX-1 antibody.

7. Preliminary experiments indicated that a single intraperitoneal (IP) injection of 400 µg LPS in wildtype BALB/c mice resulted in a 80% mortality rate within 2 days. In a first experiment using the antibodies, four groups of BALB/c mice (n=8 in each group) were therefore treated by a single IP injection of antibody KRIX-1 or of its deglycosylated form (KRIX-1Q) prior to administration of 400 µg LPS. Mouse survival was followed over time. The results on the prevention and/or treatment of sepsis in this experiment are illustrated in Figure 1A. This Figure shows that all mice survived endotoxin-mediated shock upon treatment with 3 or 30 µg of KRIX-1 or 3 µg of KRIX-1Q. A significant improvement of survival rate was also observed at a dose of 0.3 µg KRIX-1.

8. In another experiment, wildtype C57B1/6 mice were injected with KRIX-1Q (30 µg, 3 µg, or 0.3 µg), a sham IgG4 antibody (AK6A3), or buffer. Thirty minutes later a single IP injection of 400 µg LPS was administered. Survival of the mice was subsequently monitored. When mice were administered the KRIX-1Q antibody an anti-sepsis response was observed. In particular, it was observed that the KRIX-1Q antibody can be administered in high dosages without occurrence of shock as a result of pro-inflammatory and anti-inflammatory compensatory responses as observed with complete inhibition of FVIII. The effectiveness against sepsis is illustrated in Figure 1B. This Figure shows a statistically significant death prevention with 30 µg of KRIX-1Q compared to mice receiving either the sham IgG4 (AK6A3) ($p<0.03$) antibody or no antibody (i.e., buffer).

9. The results illustrated in Figure 1A and 1B clearly demonstrate that the KRIX-1 and KRIX-1Q antibodies are effective against sepsis in the mouse model.

10. To further demonstrate that partially inhibitory antibodies directed against the C1 domain of FVIII are readily obtained following the methods described in the specification of our patent application, we present the following data in connection with the antibody named RHD5.

11. In general, a human lymphoblastoid cell line, named RHD5, was derived by immortalization of B lymphocytes from a patient with acquired hemophilia, as described in the specification. These B cells were then transformed by infection with Epstein-Barr virus as follows. Briefly, 10^7 peripheral blood mononuclear cells were resuspended in 2 mL culture medium and incubated for 2 hours at 37°C with 200 µL Epstein-Barr virus supernatant (B95-8 strain). Cells were then seeded at 5,000 cells/well in 96-well microtiter plates (Nunc) containing feeder cells (3T6-TRAP cells irradiated with 7,000 rads). One hundred fifty microliters of culture supernatant was replaced every week by fresh culture medium.

12. After 6 weeks, culture supernatants were tested in an enzyme-linked immunosorbent assay (ELISA) for the presence of anti-FVIII antibodies. Positive cell lines were transferred to 24-well plates and immediately cloned at 60 cells per 96-well plate without feeder cells. One clone, producing an antibody called RHD5, was selected. The antibody present in the culture supernatant was purified by adsorption on HiTRAP protein A (Pharmacia), as described in the specification.

13. The fact that RHD5 binds to the C1 domain of FVIII, similar to KRIX-1 was confirmed by immunoreactivity to FVIII fragments corresponding to the C1 domain of FVIII.

14. Inhibitory activity or RHD5 was assessed in a Bethesda assay. RHD5 inhibited only partially FVIII activity up to the highest concentration tested. In a Bethesda assay performed by mixing one volume of antibody at 200 microgram/mL or of control buffer with one volume of plasma, the residual FVIII levels were 7.0 ± 0.2 and 251.9 ± 18.8 ng/mL, respectively (mean ± SD of triplicates). RHD6 (at a final concentration of 100 µg/mL) inhibited FVIII by at least 97%. Similarly, in a Bethesda assay performed by mixing one volume of RHD5 antibody at 200 microgram/mL or of control buffer with one volume of full length recombinant FVIII (Recombinate^R, Baxter), the residual FVIII levels were 6.0 ± 0.2 and 399.7 ± 18.8 ng/mL, respectively (mean ± SD of triplicates). The inhibition of FVIII activity reached at a final concentration of RHD6 of 100 microgram/mL was therefore 98%. A dose response curve of plasma FVIII inhibition by RHD5 is shown in Figure 2.

15. The ability of KRIX-1 to compete with RHD5 for FVIII binding was also tested in ELISA. Polystyre microtitration plates were incubated overnight at 4°C with 50 µL RHD5 at 2 microgram/mL in phosphate buffered saline (PBS). The plates were next washed 4 times with PBS-Tween. Biotinylated recombinant FVIII (0.5 microgram/mL) in Tris-BSA-Tween was mixed with RHD5 or KRIX-1 at various concentrations before addition to RHD5 coated plates. After a two hour incubation period at 4°C, the plates

were washed 4 times and bound biotinylated FVIII was detected by addition of avidine peroxidase (Sigma) at 1 microgram/mL. After 30 minutes at room temperature (RT), the plates were washed again and supplemented with 100 µL OPD. The resulting OD was read at 490 nm in a Emax Microplate Reader (Molecular Devices, Menlo Park, CA). Biotinylated FVIII used in the above experiment was prepared by incubating recombinant FVIII (100 microgram/mL) dialysed in Hepes buffer (Hepes 10 mM, NaCl 0,15 M, $CaCl_2$ 10 mM, pH 6.5) with sulfo-NHS-LC-biotin (Pierce) at 1 microgram/mL for 2 hours at RT. The preparation was then dialysed against Hepes buffer and stored and –80°C.

16. As shown in Figure 3, KRIX-1 completely prevented FVIII binding to RHD5. These data confirm that RHD5, like KRIX-1, is directed against the C1 domain of FVIII.

17. I note that these data support the fact that antibodies such as KRIX-1 and RHD5 directed against the C1 domain of factor VIII and capable of partially inhibiting FVIII are indicative of results which can be obtained following the methods described in the application.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: OCTOBER 13 2005

Dr. Jean-Marie Saint-Remy

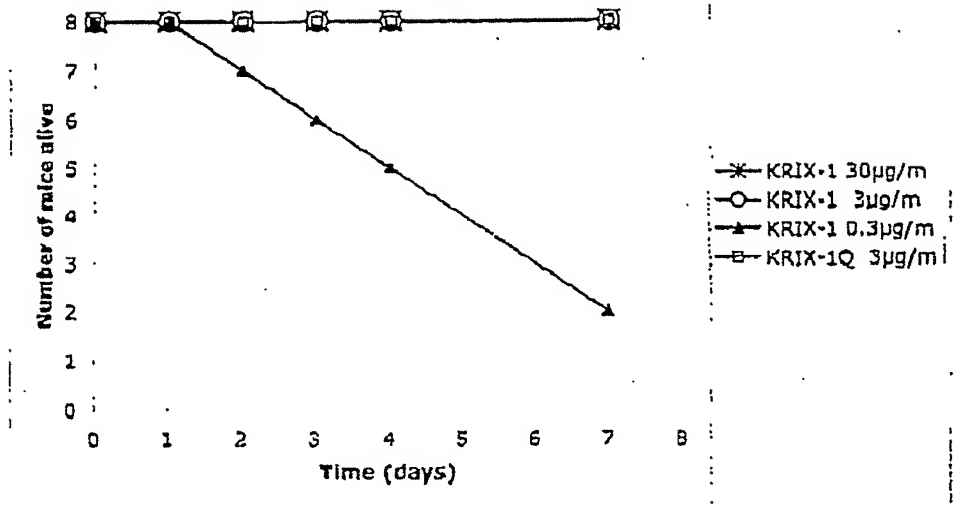

Figure 1A: Survival in a septic shock model of mice upon co-administration of LPS with partial inhibitory antibodies against Factor VIII

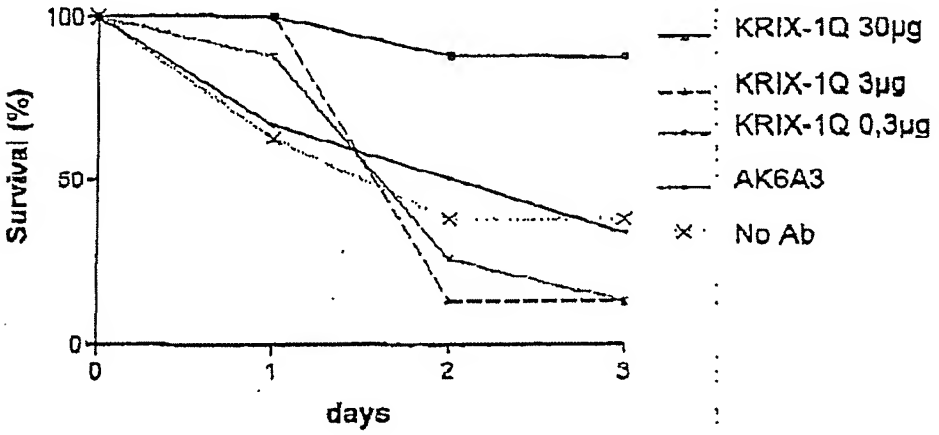

Figure 1B: Survival in a septic shock model of mice pretreated with partial inhibitory antibodies against Factor VIII.

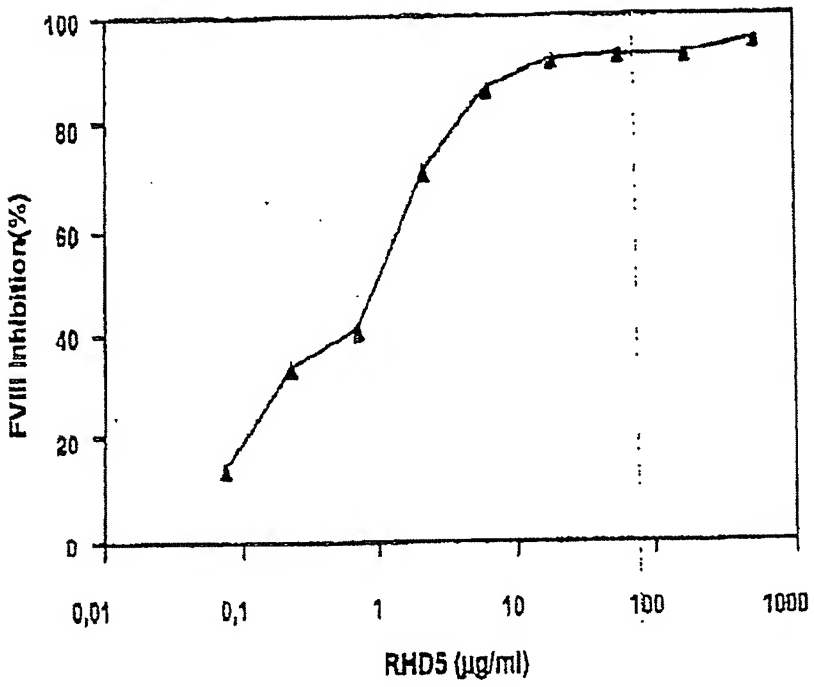

Figure 2: Dose response curve of plasma FVIII inhibition by RHD5

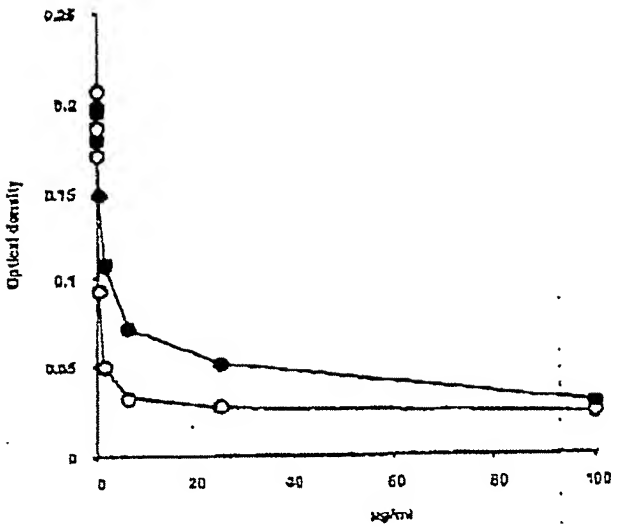

Figure 3: Competition of RHD5 and Krix-1 for the binding to C1 domain of FVIII. Different concentrations of RHD5 (closed symbols) or Krix-1 (open symbols) were mixed with rFVIII before addition to RHD5 coated plates. The plates were then incubated for 2 hours at 4°C and the binding of FVIII was detected by the addition of avidine peroxidase and OPD.

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Curriculum vitae, June 2005

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Appointments within the University of Leuven

1995-1996	Research Associate
1996-1999	Docent (Assistant Professor)
1999- present	Hoofddocent (Associate Professor)

Academic Appointments outside the University of Leuven

1982-1989	Senior Investigator, Institute of Cellular and Molecular Pathology Université de Louvain, Brussels, Belgium
1989-1995	Research Director, Allergy and Clinical Immunology Unit, Université de Louvain, Brussels, Belgium

Other Activities

1993-2002	President of the Belgian Society for Allergy and Clinical Immunology
1996-	Consultant, Allergy and Clinical Immunology, Institut Edith Cavell, Brussels, Belgium

Awards and Honors

1980-81	Fellowship of the International Institute for Molecular and Cellular Pathology (ICP, Brussels, Belgium)
1983-84	Pharmacia Award for Allergy and Clinical Immunology
1989	de Hovre Foundation Award for Immunology
2003-2005	Bayer International Award for Haemophilia basic research
2005-2007	Bayer International Award for Haemophilia special projects

Membership in Scientific Organizations

1980	Belgian Society for Allergy and Clinical Immunology
1992	British Society for Allergy and Clinical Immunology
1988	European Academy for Allergy and Clinical Immunology
1988	International Association for Allergy and Clinical Immunology
1993	Belgian Society for Thrombosis and Haemostasis
1994	Société belge d'Oto-Rhino-Laryngologie
1994	European Ligand Association
1997	American Society of Hematology
1999	International Society for Thrombosis and Haemostasis
2000	Collegium Internationale Allergologicum

Publications

Author on over 100 papers published in international peer-reviewed journals, of which a selection is provided herunder.

1. Saint-Remy JM, Lacroix-Desmazes S, Oldenburg J. Inhibitors in haemophilia: pathophysiology. Haemophilia. 2004 Oct;10 Suppl 4:146-51. Review.

2. Pipe SW, Saint-Remy JM, Walsh CE. New high-technology products for the treatment of haemophilia. Haemophilia. 2004 Oct;10 Suppl 4:55-63. Review.

3. Jacquemin M, Saint-Remy JM. The use of antibodies to coagulation factors for anticoagulant therapy. Curr Med Chem. 2004 Sep;11(17):2291-6. Review.

4. Jacquemin MG, Saint-Remy JM. Factor VIII alloantibodies in hemophilia. Curr Opin Hematol. 2004 May;11(3):146-50. Review.

5. Gilles JG, Grailly SC, De Maeyer M, Jacquemin MG, VanderElst LP, Saint-Remy JM. In vivo neutralization of a C2 domain-specific human anti-Factor VIII inhibitor by an anti-idiotypic antibody. Blood. 2004 Apr 1;103(7):2617-23. Epub 2003 Dec 11.

6. d'Oiron R, Lavergne JM, Lavend'homme R, Benhida A, Bordet JC, Negrier C, Peerlinck K, Vermylen J, Saint-Remy JM, Jacquemin M. Deletion of alanine 2201 in the FVIII C2 domain results in mild hemophilia A by impairing FVIII binding to VWF and

phospholipids and destroys a major FVIII antigenic determinant involved in inhibitor development. Blood. 2004 Jan 1;103(1):155-7. Epub 2003 Sep 11.

7. Dewerchin M, Van der Elst L, Singh I, Grailly S, Saint-Remy JM, Collen D, Jacquemin M. Inhibition of factor VIII with a partially inhibitory human recombinant monoclonal antibody prevents thrombotic events in a transgenic model of type II HBS antithrombin deficiency in mice. J Thromb Haemost. 2004 Jan;2(1):77-84.

8. Janssens W, Carlier V, Wu B, VanderElst L, Jacquemin MG, Saint-Remy JM. CD4+CD25+ T cells lyse antigen-presenting B cells by Fas-Fas ligand interaction in an epitope-specific manner. J Immunol. 2003 Nov 1;171(9):4604-12.

9. Raut S, Villard S, Grailly S, Gilles JG, Granier C, Saint-Remy JM, Barrowcliffe TW. Anti-heavy-chain monoclonal antibodies directed to the acidic regions of the factor VIII molecule inhibit the binding of factor VIII to phospholipids and von Willebrand factor. Thromb Haemost. 2003 Sep;90(3):385-97.

10. Villard S, Lacroix-Desmazes S, Kieber-Emmons T, Piquer D, Grailly S,Benhida A, Kaveri SV, Saint-Remy JM, Granier C. Peptide decoys selected by phage display block in vitro and in vivo activity of a human anti-FVIII inhibitor. Blood. 2003 Aug 1;102(3):949-52. Epub 2003 Apr 3.

11. Jacquemin M, De Maeyer M, D'Oiron R, Lavend'Homme R, Peerlinck K, Saint-Remy JM. Molecular mechanisms of mild and moderate hemophilia A.J Thromb Haemost. 2003 Mar;1(3):456-63. Review. Erratum in. J Thromb Haemost. 2003 Dec;1(12):2722.

12. Jacquemin M, Vantomme V, Buhot C, Lavend'homme R, Burny W, Demotte N, Chaux P, Peerlinck K, Vermylen J, Maillere B, van der Bruggen P, Saint-Remy JM. CD4+ T-cell clones specific for wild-type factor VIII: a molecular mechanism responsible for a higher incidence of inhibitor formation in mild/moderate hemophilia A. Blood. 2003 Feb 15;101(4):1351-8. Epub 2002 Oct 17.

13. Janssens W, Chuah MK, Naldini L, Follenzi A, Collen D, Saint-Remy JM, VandenDriessche T. Efficiency of onco-retroviral and lentiviral gene transfer into primary mouse and human B-lymphocytes is pseudotype dependent. Hum Gene Ther. 2003 Feb 10;14(3):263-76.

14. Kallas A, Pooga M, Benhida A, Jacquemin M, Saint-Remy JM. Epitope specificity of anti-FVIII antibodies during immune tolerance therapy with factor VIII preparation containing von Willebrand factor. Thromb Res. 2002 Sep 15;107(6):291-302.

15. Wu B, Elst LV, Carlier V, Jacquemin MG, Saint-Remy JM. The Dermatophagoides pteronyssinus group 2 allergen contains a universally immunogenic T cell epitope. J Immunol. 2002 Sep 1;169(5):2430-5.

16. Behrmann M, Pasi J, Saint-Remy JM, Kotitschke R, Kloft M. Von Willebrand factor modulates factor VIII immunogenicity: comparative study of different factor VIII concentrates in a haemophilia A mouse model. Thromb Haemost. 2002 Aug;88(2):221-9.

17. Saint-Remy JM. Immunology of factor VIII inhibitors. Semin Thromb Hemost. 2002 Jun;28(3):265-8. Review.

18. Singh I, Smith A, Vanzieleghem B, Collen D, Burnand K, Saint-Remy JM, Jacquemin M. Antithrombotic effects of controlled inhibition of factor VIII with a partially inhibitory human monoclonal antibody in a murine vena cava thrombosis model. Blood. 2002 May 1;99(9):3235-40.

19. Saint-Remy JM. Hemophilia factor VIII therapy. B- and T-cell tolerance from basic concepts to clinical practice. Haematologica. 2000 Oct;85(10 Suppl):93-6. Review.